Ibotenic Acid Analogues. Synthesis and Biological Testing of Two Bicyclic 3-Isoxazolol Amino Acids

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The bicyclic 3-isoxazolol amino acids (RS)-3-hydroxy-4,5,6,7-tetrahydroisoxazolol[4,5-c]pyridine-4-carboxylic acid (5, 4-HPCA) and (RS)-3-hydroxy-4,5,6,7-tetrahydroisoxazolol[4,5-c]pyridine-6-carboxylic acid (11, 6-HPCA) were synthesized as model compounds for studies of the structural requirements of central excitatory amino acid neurotransmitter receptors. 4-HPCA was synthesized via introduction of a methoxy carbonyl group into the 4-position of the lithiated N-nitroso intermediate J. The key reaction in the synthesis of 6-HPCA is an intramolecular N-alkylation of the appropriately substituted acetamidomalonate derivative 7 using sodium hydride as a base. On the basis of the pK₄ values for 4-HPCA the existence of an intramolecular hydrogen bond in the zwitterionic form of this amino acid is proposed. 6-HPCA was shown by 1H NMR spectroscopy to adopt preferentially a conformation with the carboxylate group in an equatorial position. 4- and 6-HPCA were tested as agonists and antagonists at excitatory amino acid receptors on neurones in the cat spinal cord using microelectrophoretic techniques. Neither compound showed significant effects at these receptors.

Accumulating evidence strongly suggests that the amino acids (S)-glutamic acid (GLU) and (S)-aspartic acid (ASP) are the major excitatory neurotransmitters in the mammalian central nervous system. The possible involvement of these amino acid transmitters in certain neurological diseases has focused much interest on the central excitant amino acid receptors as sites for pharmacological and therapeutic attack. Electrophysiological and receptor binding studies have disclosed heterogeneity of these receptors, which at present are most conveniently subdivided into three classes: 1. Quisqualic acid (QUIS) receptors, at which the naturally occurring amino acid QUIS is a selective agonist; 2. N-methyl-(R)-aspartic acid (NMA) receptors, at which NMA is a powerful agonist and a number of compounds, including 2-amino adipic acid, are antagonists (Scheme 1); 3. Kainic acid receptors.

The QUIS receptors probably represent the postsynaptic GLU receptors. Using the naturally occurring amino acid ibotenic acid as a lead structure, structure-activity studies on a number of specific QUIS receptor agonists such as AMPA and 5-HPCA (Scheme 1), have shed light on the “receptor-active conformation” of GLU at these receptors.

The NMA receptors are assumed to represent primarily central ASP receptors. However, very little is known about the “receptor-active conformation” of ASP and NMA and the conformation, in which 2-amino adipic acid binds to and blocks these receptors. As part of our current studies of these aspects we now report the synthesis of (RS)-3-hydroxy-4,5,6,7-tetrahydroisoxazolol[4,5-c]pyridine-4-carboxylic acid (5, 4-HPCA) and (RS)-3-hydroxy-4,5,6,7-tetrahydro-
isoxazol[4,5-c]pyridine-6-carboxylic acid (II, 6-HPCA), which are conformationally restricted analogues of NMA and 2-aminoadipic acid, respectively (Scheme 1).

Results and discussion

Since ibotenic acid is relatively easily decarboxylated, and 4-HPCA in analogy to ibotenic acid has a carboxylate group in a position α to the isoxazole ring (Scheme 1), a reaction sequence for the synthesis of 4-HPCA (5) was developed, in which the carboxylate group was unmasked under mild conditions in the last step (Scheme 2). A methoxycarbonyl group was introduced regio-specifically into the 4-position of 1 under strongly basic conditions. Attempts to substitute other bases, including sodium hydride or potassium tert-butyllithium, for butylthiophenium to give detectable amounts of 2. The stepwise deprotection of 2 was initiated by passing a stream of hydrogen bromide gas through a solution of 2 in glacial acetic acid, until the formation of a brown volatile product, assumed to be nitrosoyl bromide, ceased. The 3-methoxy group of 3 was selectively cleaved by treatment of 3 with a concentrated solution of hydrogen bromide in glacial acetic acid, whereas the conversion of 4 into 5 was accomplished by treatment of 4 with an aqueous solution of triethylamine.

Treatment of 6 with equimolar amounts of N-bromosuccinimide (NBS) gave 7 contaminated with very small amounts of the dibromo compound 8. A separable mixture of 7 and 8 was obtained by using NBS in excess. Compound 8 is a key intermediate in the synthesis of other compounds related to ibotenic acid. Cyclization of 7 into 9 was accomplished using sodium hydride as a base. The reaction product obtained after demethylation of 10 using hydrogen bromide in glacial acetic acid gave after treatment with water the zwitterion II rather than its hydrobromide. This observation probably is the result of the exceptionally low $pK_A$ value (<1.2) of II.

The structures of the new compounds 2–5 and 7–11 were established on the basis of elemental analyses, IR, and $^1$H NMR spectroscopic data, and, in the case of 2, by mass spectrometry. In agreement with the findings for I,15 two sets of resonance signals were detected in the $^1$H NMR spectrum of 2 due to hindered rotation around the N–N bond. A comparison of the $pK_A$ values of 5 (1.6, 5.8, 8.5) with those of II (<1.2, 4.2, 8.2), ibotenic acid (3, 5.0, 8.2),16 AMPA (2.5, 4.8, 10.0), and 5-HPCA (2.2, 4.7, 8.1) reveals a strikingly large difference between the $pK_A$ I and II values of 5. This difference is interpreted in terms of the existence of an intramolecular hydrogen bond7 between the carboxylate and hydroxy groups of 5 (Scheme 1). There is no obvious explanation of the exceptionally low $pK_A$ I value of II.

The $^1$H NMR spectrum of II shows coupling constants between the C-6 proton and the two C-7 protons ($J_{	ext{H6,H7c}} = 10$ Hz, $J_{	ext{H6,H7a}} = 5$ Hz) in accordance with axial-pseudoaxial and axial-pseudoequatorial configurations, respectively, of these protons. This is consistent with a predominantly equatorial orientation of the carboxylate group at C-6. Long-range couplings between the C-4 and C-7 protons ($J = 0.5, 2, 2,$ and 2 Hz) were detected in the $^1$H NMR spectrum of II.

The effects of 5 (4-HPCA) and II (6-HPCA) on cat spinal neurones were tested using microelectrophoretic techniques.18 However, neither 4-nor 6-HPCA showed any significant excitatory effects on these neurones, and neither compound was capable of reducing significantly the excitatory effects of QUIS, NMA, or kainic acid. These observations seem to indicate that 4-HPCA, designed as a conformationally restricted analogue of NMA (Scheme 1), does not reflect the active conformation of NMA at its receptors. Similarly, the inactivity of 6-HPCA has been interpreted in terms of 2-aminoadipic acid adopting a conformation different from that reflected by its conformationally restricted analogue 6-HPCA (Scheme 1) during its binding to and blockade of the NMA receptors.

Experimental

Melting points are corrected and were determined in capillary tubes. Elemental analyses were performed by Mr. P. Hansen, Chemical Laboratory II, University of Copenhagen. IR spectra, obtained on a Perkin-Elmer Grating Infrared Spectrophotometer, model 247, were recorded in KBr pellets. The 60 MHz $^1$H NMR spectra (compounds 2–4 and 7–10) were recorded at 25°C on a Varian 360L spectrometer. The 270 MHz spectra (compounds 5 and 11) were recorded on a Bruker HX 270 S instrument. Fourier transform method was used to obtain the
spectra with spectral widths of 3000 or 5000 Hz with digital resolution of 0.3 Hz. TMS was used as an internal standard except for the compounds dissolved in D₂O, where 3-(trimethylsilyl)propanesulphonate was used. Thin layer chromatography (TLC) and gravity column chromatography (CC) were performed using silica gel F₂₅₄ plates (Merck) and silica gel (Woclm, 0.063–0.200 mm), respectively. Compounds containing the 3-isoxazolol unit were visualized on TLC plates using UV light and a FeCl₃ spraying reagent (yellow colour). Compounds containing amino groups were visualized using a ninhydrin spraying reagent, and all compounds under study were detected on TLC plates using a K₂MnO₄ spraying reagent. All evaporation were performed at ca. 15 mm Hg using a rotatory evaporator. The pKₐ values were determined using a published procedure,¹⁰,²⁰ except that the titration was carried out with 0.1 N HCl or 0.1 N NaOH. The ionic strength was kept constant using 0.15 M KCl.

(RS)-Methyl 3-methoxy-5-nitroso-4,5,6,7-tetrahydroisoxazolol[4,5-c]pyridine-4-carboxylate (2). To a stirred solution of 1¹⁵ (1.00 g; 5.5 mmol) in dry tetrahydrofuran (THF) (20 ml), kept under a ni-

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Scheme 1.

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Scheme 2.
trogen blanket at ca. −75°C, was added butyl-lithium (4.0 mL of a 1.5 M solution in hexane; 6.0 mmol), and after 1 min methyl chloroformate (4.2 mL; 55 mmol) was added during a period of 1 min. Stirring was continued at ca. −75°C for 5 min, and upon addition of glacial acetic acid (0.2 mL) the solution was evaporated. Water (15 mL) was added to the residue and the mixture extracted with dichloromethane (3×20 mL). The combined and dried (MgSO₄) organic phases were evaporated. CC [silica gel; 100 g; eluents: dichloromethane containing ethyl acetate (5–10%)] gave 2 (208 mg; 16%), m.p. 99.5–100.5°C (ethyl acetate-light petroleum). Anal. C₈H₁₃N₂O₆; C, H, N. IR: 3400 (w), 2995 (w), 2960 (w), 2930 (w), 1745 (s), 1660 (m), 1525 (s) cm⁻¹. ¹H NMR (CDCl₃): δ 6.4 (0.2 H, s), 6.2 (0.8 H, s), 5.4–5.0 (1 H, m), 4.7–4.2 (1 H, m), 4.2 (ca. 0.6 H, s), 4.1 (ca. 2.4 H, s), 3.9 (ca. 0.6 H, s), 3.8 (ca. 2.4 H, s), 3.3–2.8 (2 H, m). MS [70 eV; m/z (% rel. int.)]: 241 (7, M), 222 (6), 211 (39), 182 (100), 152 (73), 137 (31), 121 (25), 59 (29).

(RS)-Methyl 3-methoxy-4,5,6,7-tetrahydroisoxazol[4,5-c]pyridine-4-carboxylate Hydrobromide (3). Through a solution of 2 (200 mg; 0.83 mmol) in glacial acetic acid (10 mL), kept at 0°C, was passed a stream of hydrogen bromide gas, until the solution became pale yellow (60 s). The solution was immediately evaporated and the residue recrystallized (methanol-ether) to give 3 (224 mg; 92%), m.p. 143–144°C (decomp.). Anal. C₁₅H₂₃N₂O₄Br: C, H, Br; C: Calcd. 36.88; found 36.00. IR: 3600–3300 (w), 3010–2400 (several bands, m–s), 1745 (s), 1665 (s), 1545 (s), 1525 (s) cm⁻¹. ¹H NMR (D₂O): δ 5.5 (1 H, s), 4.1 (3 H, s), 4.0 (3 H, s), 4.0–3.6 (2 H, m), 3.3–3.0 (2 H, m).

(RS)-Methyl 3-hydroxy-4,5,6,7-tetrahydroisoxazol[4,5-c]pyridine-4-carboxylate Hydrobromide (4). A solution of 3 (100 mg; 0.34 mmol) in a solution of hydrogen bromide in glacial acetic acid (5 mL; 33%) was kept at 25°C for 18 h. The solution was evaporated and the residue recrystallized (methanol-ether) to give 4 (85 mg; 89%), m.p. 141–143°C (decomp.). Anal. C₁₆H₂₅N₂O₅Br: C, H, N, Br; IR: 3600–3300 (w), 3200–2400 (several bands, w–m), 1745 (s), 1665 (m), 1565–1500 (several bands, m) cm⁻¹. ¹H NMR (D₂O): δ 5.5 (1 H, s), 4.0 (3 H, s), 4.0–3.6 (2 H, m), 3.3–3.0 (2 H, m).
boxylate (9). To a suspension of sodium hydride (18.2 mg; 0.76 mmol) in dry N,N-dimethylformamide (2.5 ml), kept at ca. -15°C, was added, during a period of 30 min, 7 (160 mg; 0.38 mmol). After stirring for an additional 30 min at ca. -10°C glacial acetic acid (0.2 ml) was added and the solution evaporated. Upon addition of water (5 ml) the mixture was extracted with chloroform (3 x 10 ml). The combined chloroform phases were dried (MgSO₄) and evaporated to give an oil. CC [silica gel: 7 g; eluents: dichloromethane containing butanone (5-10 %)] gave 9 (84 mg; 65 %), m.p. 89.0–90.0°C (ethyl acetate-light petroleum). Anal. C₁₅H₂₁N₂O₇; C, H, N. IR: 3600–3320 (w), 3020 (w), 2990 (m), 1745 (s), 1730 (s), 1690 (s), 1680 (s), 1530 (s) cm⁻¹. ¹H NMR (CDCl₃): δ 4.5 (2 H, s), 4.3 (4 H, q, J 7 Hz), 4.0 (3 H, s), 3.5 (2 H, s), 2.2 (3 H, s), 1.3 (6 H, t, J 7 Hz).

(RS)-3-Methoxy-4,5,6,7-tetrahydroisoxazolo-[4,5-c]pyridine-6-carboxylic acid hydrochloride (10). A suspension of 9 (150 mg; 0.44 mmol) in hydrochloric acid (10 ml; 1 M) was heated to reflux for 2.5 h. After cooling to 25°C and filtration the solution was evaporated to dryness and the residue recrystallized (methanol-ethyl acetate) to give 10 (57 mg; 55 %), m.p. 220–224°C (decomp.). Anal. C₁₃H₁₃N₂O₂Cl; C, H, N; Cl; calcld, 15.11; found, 12.92. IR: 3600–3300 (w), 3200–2300 (m-s), 1750 (s), 1675 (m), 1570 (w), 1520 (s) cm⁻¹. ¹H NMR (D₂O): δ 4.4 (1 H, m), 4.2 (2 H, s), 3.9 (3 H, s), 3.3 (2 H, m).

(RS)-3-Hydroxy-4,5,6,7-tetrahydroisoxazolo-[4,5-c]pyridine-6-carboxylic acid zwitterion. 1/4 H₂O (6-HPCA) (11). A solution of 10 (30 mg; 0.13 mmol) in a solution of hydrogen bromide in glacial acetic acid (1.5 ml; 33 %) was kept at 25°C for 18 h and then evaporated. The residue was dissolved in water (15 ml) and the solution evaporated. Recrystallization (water) of the residue gave 11 (18 mg; 75 %), m.p. >350°C. Anal. C₁₅H₁₅N₂O₄ · 0.45 H₂O; C, H, N. IR: 3600–3300 (m), 3115 (s), 2970 (w), 2840–2300 (several bands, w-m), 1670 (s), 1650–1500 (several bands, m-s) cm⁻¹. ¹H NMR (sodium carbonate in D₂O, ca. 1 M): δ 3.61 (1 H, broad dd, J 15, 2, and 0.5 Hz), 3.49 (1 H, dt, J 15, 2, and 2 Hz), 3.47 (1 H, dd, J 10 and 5 Hz), 2.83 (1 H, broad dq, J 16.5, 5, 2, and 0.5 Hz), 2.57 (1 H, ddt, J 16.5, 10, 2, and 2 Hz). pKₐ values (H₂O, 25°C): <1.2, 4.2, 8.2.

pKₐ Values for (RS)-a-amino-3-hydroxy-5-methylisoxazole-4-propionic Acid (AMPA) and (RS)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridine-5-carboxylic acid (5-HPCA). AMPA (H₂O, 25°C): 2.5, 4.8, 10.0. 5-HPCA (H₂O, 25°C): 2.2, 4.7, 8.1.

Microelectrophoretic studies. Experiments were performed on lumbar dorsal horn interneurones and Renshaw cells of cats anaesthetized with pentobarbitone sodium (35 mg/kg intraperitoneally initially supplemented intravenously when required). Extracellular action potentials were recorded by means of the centre barrel of seven-barrel micropipettes, which contained 3.6 M NaCl. The compounds were administered electrophoretically from the outer barrels of the micropipettes, which contained aqueous solutions: NMA (0.05 M in 0.15 M NaCl, pH 7.6), QUIS (0.005 M in 0.15 M NaCl, pH 7.5), 4-HPCA (0.1 M, pH 7.3), and 6-HPCA (0.1 M, pH 7.3). The excitatory amino acids were administered for times sufficient to obtain maximal effects at the particular rate of ejection.

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